

Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World

Marc A. McPherson¹, Allen G. Good², A. Keith C. Topinka³, and Linda M. Hall^{1,4}

¹Alberta Agriculture, Food and Rural Development, 410 Agriculture/Forestry Building, University of Alberta, Edmonton, Alberta, Canada T6G 4P2 (linda.hall@gov.ab.ca); ²Department of Biological Sciences, CW405 Biological Sciences Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2E9 (allen.good@ualberta.ca); ³Crop Diversification Centre North, 17507 Fort Road N. W., Edmonton, Alberta, Canada T5Y 6H3 (keith.topinka@gov.ab.ca).
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McPherson, M. A., Good, A. G., Topinka, A. K. C. and Hall, L. M. 2004. **Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World.** Can. J. Plant Sci. **84**: 923–934. *Carthamus tinctorius* L. (safflower) is being evaluated as a crop for the production of plant-made pharmaceuticals using an oleosin fusion protein system. We evaluated the potential for transgenic gene flow from *C. tinctorius* to wild or weedy relatives in western Canada. Cytogenetic and phylogenetic studies with most of the species of *Carthamus* have demonstrated that cultivated *C. tinctorius* has the ability to hybridize with at least six wild or weedy relatives worldwide. Of the four naturalized wild relatives in the New World, only two, *C. oxyacanthus* and *C. creticus*, have successfully been crossed with *C. tinctorius* to produce fertile hybrids. Data from artificial crosses resulting in fertile offspring indicate the biological potential of a hybridization event, but only if the species are temporally and spatially sympatric can this occur. Based on the New World distribution of *C. oxyacanthus* and *C. creticus* we predict that hybridization with transgenic *C. tinctorius* could occur in some areas of Argentina, Chile and localities within several states in the United States including California, Florida, Illinois, Kansas, New Mexico, Ohio, Oklahoma, Oregon, Utah, and Texas. Locations in the New World where wild species of *Carthamus* have not been naturalized may provide biologically isolated locations for the cultivation of a transgenic safflower crop.

Key words: *Carthamus*, safflower, transgenic, hybrid, gene flow, introgression.

McPherson, M. A., Good, A. G., Topinka, A. K. C. et Hall, L. M. 2004. **Potential d'hybridation théorique du carthame transgénique (*Carthamus tinctorius* L.) avec les adventices apparentées d'Amérique.** Can. J. Plant Sci. **84**: 923–934. On s'intéresse au carthame (*Carthamus tinctorius* L.) pour la production de substances pharmaceutiques végétales par le biais d'un système de protéines hybrides de l'oléosine. Les auteurs ont tenté d'évaluer les risques de flux génétique entre le carthame et les espèces sauvages ou adventices apparentées qu'on trouve dans l'ouest du Canada. Les études cytogénétiques et phylogénétiques sur la majorité des espèces du genre *Carthamus* indiquent que le carthame pourrait se reproduire avec au moins six espèces sauvages ou adventices dans le monde. Sur les quatre qui se sont acclimatées en Amérique, seules *C. oxyacanthus* et *C. creticus* ont donné des hybrides fertiles après croisement avec *C. tinctorius*. Les données sur les croisements qui ont abouti à des hybrides fertiles nous renseignent sur le potentiel biologique d'hybridation, mais un croisement ne peut survenir que si les deux espèces sont sympatriques dans le temps et l'espace. Compte tenu de l'aire de distribution de *C. oxyacanthus* et de *C. creticus* en Amérique, les auteurs estiment qu'il pourrait y avoir hybridation dans certaines parties de l'Argentine, du Chili et de plusieurs États américains, dont la Californie, la Floride, l'Illinois, le Kansas, le Nouveau-Mexique, l'Ohio, l'Oklahoma, l'Orégon, l'Utah et le Texas. Les endroits d'Amérique où les espèces sauvages du genre *Carthamus* ne se sont pas acclimatées pourraient constituer des endroits biologiquement isolés où l'on pourrait cultiver le carthame transgénique.

Mots clés: *Carthamus*, carthame, transgénique, hybride, flux génétique, introgression

Safflower (*Carthamus tinctorius* L.), one of humanity's oldest crops (Johnston et al. 2002), is currently being evaluated as a host for a new transgenic technology, plant-made protein-based pharmaceuticals (Lacey et al. 1998). Safflower was originally grown to produce dyes (carthamine) for food and fabric and for medicinal use, but is currently cultivated for edible oil and birdseed. Annual world production of safflower is estimated at 800 000 t, (Gyulai 1996), not including a large number of small garden plots throughout India

and Pakistan harvested for local use (Johnston et al. 2002). Safflower is thought to have originated in the Euphrates basin (Knowles 1969, 1989) and from this center of origin, cultivation expanded to Egypt, Ethiopia, southern Europe, southern Asia and the Far East (Smith 1996). Hybridization with several wild species of *Carthamus* may have played a major role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (Ashri and Knowles 1960; Schank and Knowles 1964). Cultivation of safflower in the New World began in 1899, and it was commercially grown in the 1950s (Knowles 1958, 1989).

⁴To whom correspondence should be addressed.

Weedy and wild relatives of *C. tinctorius* were naturalized in the New World as early as 1891 (Fuller 1979).

A cultivar of *C. tinctorius* has been genetically engineered to express two novel traits. The first is a nuclear-encoded gene cassette conferring herbicide resistance. The second entailed the fusion of a gene encoding plant-made pharmaceuticals with the pre-existing gene encoding the seed protein oleosin. The utility of an oleosin fusion protein to facilitate the production of a protein of interest in an oilseed has been described previously (Parmenter et al. 1995) and the structure and isolation of the oleosin protein in safflower have been demonstrated by Lacey et al. (1998). The environmental biosafety implications of plant-made pharmaceutical production in safflower are currently being evaluated.

Gene escape from transgenic crops to wild or weedy relatives is a significant environmental safety concern. Hybridization is the first requisite step for gene escape (Rieseberg and Ellstrand 1993; Rieseberg and Wendel 1993). Species must be spatially and temporally sympatric for hybridization to occur. Introgression, the stable incorporation of a gene into a wild or weedy population, can occur if a crop/wild species hybrid successfully backcrosses to individuals of either species (Ellstrand et al. 1999; Conner et al. 2003). Hybridization and introgression between crops and their wild relatives is common and has been documented for 12 of 13 different crop species evaluated by Ellstrand et al. (1999). For example, introgression has been studied in *Oryza sativa* L. (rice; Lu et al. 2002), *Helianthus annuus* L. (sunflower; Linder et al. 1998), *Raphanus sativus* L. (cultivated radish; Snow et al. 2001), and *Phaseolus vulgaris* L. (common bean; Beebe et al. 1997). Transgenic movement to weedy or wild relatives has been documented from *Brassica napus* L. (oilseed rape; Metz et al. 1997).

Hybridization with *C. tinctorius* and several wild relatives has been demonstrated to occur artificially (hand pollination; Kadam and Patankar 1942; Claassen 1950; Ashri and Knowles 1960; Ashri and Efron 1964; Imrie and Knowles 1970; Khidir and Knowles 1970a, b; Estilai and Knowles 1976, 1978; Estilai 1977; Heaton and Klisiewicz 1981) and naturally (open pollination; Kadam and Patankar 1942; Claassen 1950; Ashri and Rudich 1965). However, cross-compatibility of species, while relatively easy to measure, is not by itself a sufficient predictor of gene flow potential under field conditions. The cultivation of safflower and naturalization of several wild relatives in the New World suggest there is a potential for hybridization between these species in areas of co-occurrence.

To assess the potential for gene escape through introgression from cultivated *C. tinctorius* (safflower) to wild relatives, the phylogenetic relationships in *Carthamus* were reviewed. Closely related species are presumably more likely to hybridize successfully, but this inference must be substantiated by empirical data derived from both open and artificial crosses. The primary source of data used to infer phylogenetic relationships in the genus *Carthamus* has been from artificial and natural interspecific hybridization experiments. The geographic distribution and biology of New World *Carthamus* species with the potential to hybridize with cultivated safflower were assessed to determine documented spatial and temporal sympatry.

Finally, we highlight directions for future research to verify the potential for interspecific gene flow from a transgenic safflower crop in North and South America.

RESULTS FROM THE LITERATURE

Taxonomy and Phylogeny

Carthamus L. is a genus of ca. 16 species *sensu* López-González (1989; Table 1). It is a member of the subtribe Centaureinae, tribe Cardueae (thistles), subfamily Tubuliflorae and family Asteraceae (Compositae; Kumar 1991; Vilatersana et al. 2000). The position of *Carthamus* in the tribe Cardueae is unclear, as is the circumscription of the genus (Vilatersana et al. 2000).

The taxonomy and classification of *Carthamus* has changed substantially as data for this diverse group have been obtained and interpreted (Table 1). To synthesize the distribution, hybridization and cytological literature for *Carthamus*, it was necessary to decipher and compare the different taxonomic schemes used by researchers over time (Table 1). For clarity, we follow the classification scheme of López-González (1989) unless otherwise stated. This classification system is based on information from morphology, biogeography, cytology and interspecific compatibility.

Delimitation of *Carthamus* and a close ally, *Carduncellus*, has been difficult due to morphological similarities and convergent evolution of several variable characters utilized by taxonomists (Vilatersana et al. 2000). These two genera constitute a large group termed the *Carduncellus*–*Carthamus* complex (Vilatersana et al. 2000). Morphological and cytological characters identified to date are insufficient to delimit the species of the *Carduncellus*–*Carthamus* complex into discrete sections and genera (Hanelt 1963; Dittrich 1969; López-González 1989; Vilatersana et al. 2000). Depending on the taxonomist and the morphological characters emphasized in their classification scheme some species in the complex have been moved in and out of *Carthamus* and *Carduncellus* (Table 1; Vilatersana et al. 2000). A new genus *Femeniasia* Susanna was recently added to the complex, and has further complicated our understanding of relationships among these closely related groups (Vilatersana et al. 2000). A recent molecular-based phylogenetic study (Vilatersana et al. 2000), several detailed morphological studies (Ashri and Knowles 1960; Hanelt 1963; López-González 1989) and cytogenetic analysis of hybrids from interspecific crosses (Ashri and Knowles 1960; Estilai and Knowles 1976; Estilai and Knowles 1978) have been used to transfer several closely related thistle species from *Carthamus* to two other genera (Table 1; *Lamottea* and *Phonus*). Five chromosome groups were identified by Ashri and Knowles (1960) in *Carthamus* ($n = 10, 11, 12, 22$ and 32) and these have influenced the delimitation of the sections of the genus (Table 1).

Vilatersana et al. (2000) analyzed DNA sequence from the Internal Transcribed Spacers of nuclear ribosomal DNA (ITS1 and ITS2) to infer the phylogenetic relationships for representatives of the *Carduncellus*–*Carthamus* complex. Vilatersana et al. (2000) obtained strong bootstrap support for the removal of *C. caeruleus* from *Carthamus* and its placement in *Lamottea* in agreement with the classification of López-González (1989;

Table 1. Comparison of the *Carthamus* intrageneric classification schemes by Hanelt (1963), Estilai and Knowles (1976) and López-González (1989)

Hanelt (1963)	Estilai and Knowles (1976)	López-González (1989)
<p>Sect. <i>Carthamus</i> (n = 12)</p> <p><i>C. curdicus</i> Hanelt</p> <p><i>C. gypsicola</i> Ilj.</p> <p><i>C. oxyacanthus</i> Bieb.</p> <p><i>C. palaestinus</i> Eig.</p> <p><i>C. persicus</i> Willd.²</p> <p><i>C. tinctorius</i> L.</p> <p>Sect. <i>Odonthagnathis</i> (n = 10)</p> <p><i>C. dentatus</i> (Forssk.) Vahl.</p> <p>spp. <i>dentatus</i> Hanelt</p> <p>spp. <i>ruber</i> (Link) Hanelt</p> <p>Sect. <i>Atractylis</i> (n = 22, 32)</p> <p><i>C. lanatus</i> L.</p> <p>spp. <i>lanatus</i> Hanelt</p> <p>spp. <i>creticus</i> (L.) Holmb.^{3,w}</p> <p>spp. <i>montanus</i></p> <p>spp. <i>turkestanicus</i> Hanelt</p> <p>Hanelt (1963)</p> <p>Sect. <i>Thamnacanthus</i></p> <p><i>C. arborescens</i> L.^y</p> <p><i>C. rhiphaeus</i> F. –Q. et Pau^s</p> <p><i>C. X martis</i> F. –Q.^s</p> <p>Sect. <i>Lepidopappus</i> Hanelt</p> <p>Series <i>Lepidopappi</i> (n = 10)</p> <p><i>C. glaucus</i> Beib.</p> <p>spp. <i>glaucus</i></p> <p>spp. <i>glandulosus</i> Hanelt</p> <p>spp. <i>anatolicus</i> Hanelt</p> <p>spp. <i>alexandrinus</i> Hanelt^y</p> <p><i>C. boissieri</i> Halácsy</p> <p><i>C. tenuis</i> (Boiss. & Bl.) Bormm.</p> <p>spp. <i>tenuis</i></p> <p>spp. <i>gracillimus</i> (Rech. f.) Hanelt</p> <p>spp. <i>foliosus</i> (Boiss.) Hanelt</p> <p>Series <i>Leucauli</i> (n = 10, 12)</p> <p><i>C. leucocaulos</i> Sibth. et Sm.</p> <p><i>C. X (?) rechingeri</i> Davis</p> <p><i>C. nitidus</i> Boiss.^u</p>	<p>Section I (n = 12)</p> <p><i>C. oxyacanthus</i> Bieb.</p> <p><i>C. palaestinus</i> Eig.</p> <p><i>C. persicus</i> Willd.²</p> <p><i>C. tinctorius</i> L.</p> <p>Section II (n = 10)</p> <p><i>C. alexandrinus</i> Ascher^y</p> <p><i>C. dentatus</i> Vahl.</p> <p><i>C. leucocaulos</i> Sibth. et Sm.</p> <p>Section III (n = 22)</p> <p><i>C. lanatus</i> L.</p> <p>Section IV (n = 11, 32)</p> <p><i>C. creticus</i> L.^w</p> <p><i>C. divaricatus</i> Beguinot & Vacc.^c</p> <p><i>C. turkestanicus</i> Popov</p> <p>Other (n = 12)</p> <p><i>C. arborescens</i> L.^y</p> <p><i>C. nitidus</i> Boiss.^u</p> <p>Estilai and Knowles (1976)</p> <p><i>Carduncellus</i> or <i>Carthamus</i></p> <p><i>C. caeruleus</i> L. (n = 12)^t</p> <p><i>Lamottea caeruleus</i> (L.) Pomel^t</p>	<p>Sect. <i>Carthamus</i> (n = 12)</p> <p><i>C. curdicus</i> Hanelt</p> <p><i>C. gypsicola</i> Ilj.</p> <p><i>C. oxyacanthus</i> Bieb.</p> <p><i>C. palaestinus</i> Eig.</p> <p><i>C. persicus</i> Willd.²</p> <p><i>C. tinctorius</i> L.</p> <p>Sect. <i>Odonthagnathis</i> (n = 10, 11)</p> <p><i>C. boissieri</i> Halácsy</p> <p><i>C. dentatus</i> (Forssk.) Vahl.</p> <p><i>C. divaricatus</i> Beguinot & Vacc.^x</p> <p><i>C. glaucus</i> Bieb.^y</p> <p><i>C. leucocaulos</i> Sibth. et Sm.</p> <p><i>C. tenuis</i> (Boiss. & Bl.) Bormm.</p> <p>Sect. <i>Atractylis</i> (n = 22, 32)</p> <p><i>C. creticus</i> L.^w</p> <p><i>C. lanatus</i> L.</p> <p><i>C. turkestanicus</i> Popov</p> <p>López-González (1989)</p> <p>Uncertain placement (n = 12)</p> <p><i>C. nitidus</i> Boiss.^u</p> <p><i>Femiasia balearica</i> Susanna</p> <p>Taxa removed from <i>Carthamus</i></p> <p><i>Phonus rhiphaeus</i> G. López^s</p> <p><i>Phonus arborescens</i> (L.) G. López^y</p>

²*C. flavescens* is a basionym for the currently recognized *C. persicus*.

³Hanelt (1963) and López-González (1989) considered *Carthamus alexandrinus* as a member of *C. glaucus*.

⁴Hanelt recognized *C. lanatus* ssp. *creticus* var. *divaricatus*, whereas Estilai & Knowles (1976) and López-González (1989) recognized *C. divaricatus*.

^w*Carthamus creticus* is a synonym of *C. baeticus* & of *C. lanatus* ssp. *creticus*.

^y*Carthamus arborescens* was included in *Phonus* by López-González (1989), and the molecular study of Vilatersana et al. (2000) provided strong evidence for the exclusion of this species from *Carthamus* and *Carduncellus*.

^u*Carthamus nitidus* was considered by López-González (1989) to constitute a possible monospecific section. Vilatersana et al. (2000) found *C. nitidus* as the sister of several species of *Odonthagnathis* justifying the placement of *C. nitidus* in either this section or in its own section.

^tLópez-González (1989) moved *Carthamus caeruleus* to the genus *Lamottea*.

^s*Carthamus rhiphaeus* Font Quer & Pau was changed to *Phonus rhiphaeus*. The hybrid *Carthamus* × *martis* Font Quer in Hanelt (1963) was considered a synonym for *Phonus arborescens* × *Phonus rhiphaeus* by López-González (1989).

Table 1). Artificial crosses with *Lamottea caeruleus* (synonym of *Carthamus caeruleus*) with other species of *Carthamus* failed to produce seed except for a single cross with *C. leucocaulos*, which produced a single sterile F₁ plant with low pollen viability (Ashri and Knowles 1960; Estilai and Knowles 1976; Estilai and Knowles 1978; Fig. 1). The poor fertility and difficulty of obtaining seeds from these crosses provides further evidence for the distant relationship of *L. caeruleus* to the other members of *Carthamus*. Phylogenetic studies using data from morphology (López-González 1989) and DNA sequences (Vilatersana et al. 2000) suggest that both *C. arborescens* and

C. rhiphaeus should be removed from *Carthamus* and placed in the genus *Phonus* (Table 1). *Phonus arborescens*, crossed with *C. divaricatus*, *C. leucocaulos*, and several *Carthamus* species with *n* = 12 (Fig. 1) did not produce seed, further establishing the distance of *P. arborescens* from the rest of the genus (Ashri and Knowles 1960; Estilai and Knowles 1976, 1978). With the transfer of these perennial taxa, *C. arborescens* and *C. rhiphaeus*, from *Carthamus*, the remaining members of the genus are annuals (Vilatersana et al. 2000).

Vilatersana et al. (2000) removed the ITS sequences obtained from three polyploid species, *C. creticus*, *C. lanatus*,

and *C. turkestanicus*, from their phylogenetic analysis because their inclusion decreased bootstrap support values. These authors interpreted this result as indicative of the hybrid origins of these species previously proposed by several authors (Ashri and Knowles 1960; Schank and Knowles 1964; Harvey and Knowles 1965; Khidir and Knowles 1970a, b; Estilai and Knowles 1978). Thus, the phylogenetic relationships of these three polyploid taxa to the other species of *Carthamus* remain obscure.

Overall the phylogenetic inference of Vilatersana et al. (2000) based on DNA sequence data is congruent with the classification of López-González based on morphological data (1989; Table 1). Further details about interspecific hybridization among the species of *Carthamus sensu* López-González (1989) are discussed and placed into an evolutionary context.

Hybridization

Interspecific Hybridization within Carthamus

Hybridization with several wild species of *Carthamus* may have played a role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (Ashri and Knowles 1960; Schank and Knowles 1964). Extensive empirical studies of interspecific hybridization of *C. tinctorius* with its wild relatives enable estimates of cross compatibility, provide information to predict potential hybridization and have been used to infer phylogenetic relationships within *Carthamus* (Kadam and Patankar 1942; Claassen 1950; Deshpande 1952; Ashri and Knowles 1960; Ashri and Efron 1964; Knowles and Schank 1964; Schank and Knowles 1964; Ashri and Rudich 1965; Knowles 1969, 1980; Imrie and Knowles 1970; Khidir and Knowles 1970a, b; Imrie and Knowles 1971; Estilai and Knowles 1976, 1978; Estilai 1977; Heaton and Klisiewicz 1981; Kumar 1991; Jambhale 1994).

Section Carthamus (Taxa with n = 12)

Several authors have considered *C. tinctorius*, *C. persicus* (syn. *C. flavescens*), *C. palaestinus* and *C. oxyacanthus* as separate species, but they are more likely races of a biological species (Table 1; Ashri and Efron 1964; Baker 1970; Imrie and Knowles 1970). The section *Carthamus sensu* López-González (1989) includes the four aforementioned species and both *C. curdicus* and *C. gypsicola* (Table 1). They share a chromosome number of $n = 12$ (Deshpande 1952; Ashri and Knowles 1960; Knowles 1989) and have been inferred as close relatives in phylogenetic studies utilizing data from morphology, cytogenetics and DNA sequences (Deshpande 1952; Ashri and Rudich 1965; Imrie and Knowles 1970; Knowles 1989; Vilatersana et al. 2000). Most species of section *Carthamus* (Table 1) have been artificially crossed to produce fertile F_1 and F_2 progeny (Figs. 1 and 2; Deshpande 1952; Ashri and Knowles 1960; Ashri and Efron 1964; Ashri and Rudich 1965; Imrie and Knowles 1970; Knowles 1989). Natural (open-pollinated) hybrids of *C. tinctorius* (BB) and *C. oxyacanthus* (BB) demonstrate hybrid vigor (Table 3; Deshpande 1952) and have been documented in both Pakistan and India where they are sympatric (Deshpande 1952; Knowles 1969; Knowles and Ashri 1995) and also when they were grown together in a green-

house (Knowles 1969; Knowles and Ashri 1995). Similar natural hybridization has been inferred between *C. tinctorius* (BB) and *C. palaestinus* (BB) in Israel, where these species are sympatric based on morphological comparisons of material from this region (Ashri and Rudich 1965; Knowles and Ashri 1995). The recent phylogenetic study of Vilatersana et al. (2000) confirmed the close relationship of three of the species from section *Carthamus* (*C. tinctorius*, *C. oxyacanthus* and *C. gypsicola*). Hybridization experiments with *C. curdicus* and *C. gypsicola* of the section *Carthamus sensu* Estilai and Knowles (1976) have not been attempted (Knowles 1989). However, the close phylogenetic relationship inferred by Vilatersana et al. (2000) for *C. gypsicola* with *C. tinctorius* and *C. oxyacanthus* might be indicative of the potential for cross-compatibility among these species.

Intersectional Crosses

Carthamus lanatus ($n = 22$; $A_1A_1B_1B_1$) is thought to have originated as a result of a hybridization event between two species (allopolyploidy), one species having a chromosome number of $n = 10$ and the other $n = 12$, followed by a subsequent doubling of the chromosomes (Table 3; Khidir and Knowles 1970b). *Carthamus lanatus* material from naturalized populations in California was examined by Ashri and Knowles (1960) and found to have regular pairing of chromosomes during meiosis. Hybrids between *C. lanatus* and species with $n = 10$ (*C. glauca*, *C. leucocaulos*, and *C. dentatus*) and $n = 12$ (*C. oxyacanthus*, *C. palaestinus*, *C. persicus*, and *C. tinctorius*) had irregular pairing of chromosomes during meiosis I and produced infertile hybrids (Table 1; Figs. 1 and 2). A hybrid from a cross of *C. dentatus* with *C. lanatus* was obtained, but the fertility of this individual was not reported (Khidir and Knowles 1970b). Attempts to observe homology of chromosomes during meiosis in hybrids with *C. lanatus* and other species of *Carthamus* have not revealed any potential parental species (Ashri and Knowles 1960; Khidir and Knowles 1970b; Estilai and Knowles 1976, 1978).

Crosses with *C. tinctorius* ($n = 12$) and *C. lanatus* ($n = 22$) produced sterile progeny (Fig. 1; Ashri and Knowles 1960; Heaton and Klisiewicz 1981). These authors reported that hybrid embryos from crosses with these two species were viable, but were unable to penetrate the pericarp during germination, preventing them from growing into fertile plants. Heaton and Klisiewicz (1981) obtained hybrids from a cross of *C. tinctorius* and *C. lanatus* when either species was used as the female recipient. Further manipulations by Heaton and Klisiewicz (1981) were required to obtain fertile hybrid plants from these crosses. They treated the rescued embryos ($n = 17$) with colchicine causing a doubling of the chromosome number and producing an autopolyploid ($n = 34$). Ashri and Knowles (1960) obtained one infertile hybrid from a cross of *C. tinctorius* and *C. lanatus* and found incomplete pairing of chromosomes during meiosis I. The sterility associated with irregular meiosis may have prevented backcrossing of these hybrids with *C. tinctorius* (Ashri and Knowles 1960; Heaton and Klisiewicz 1981). Thus, the likelihood of a hybrid between cultivated safflower and *C. lanatus* surviving under natural conditions is highly unlikely.

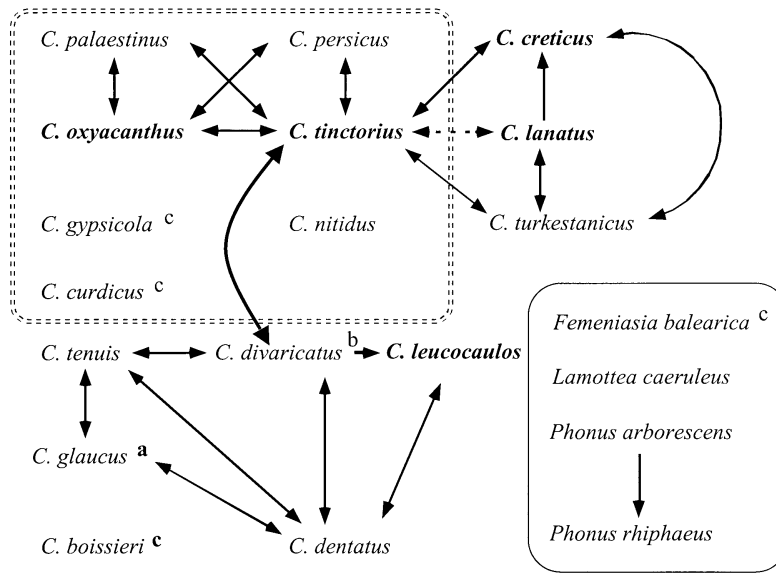


Fig. 1. Summary of the artificial interspecific crosses that resulted in fertile progeny (see text for references). Solid lines indicate fertile F_1 hybrids with viable seed production. Dotted lines indicate hybridization occurred, but the F_1 hybrids could not be obtained without embryo rescue and/or treatments with colchicine. Arrows indicate the direction of the cross (male \rightarrow female). The broken box contains taxa with $n = 12$, which are all members of section *Carthamus* except the unplaced *C. nitidus*. Species in bold are naturalized to locations in the New World. ^a *Carthamus glaucus* contains several subspecies (Table 1), which are all interfertile (Knowles and Schank 1964); however, interspecific crosses with *C. glaucus* spp. *glaucus* from different geographic regions do not produce the same results. ^b Crosses with *C. divaricatus* and *C. tinctorius* produced self-incompatible hybrids, which had low fertility when backcrossed with *C. tinctorius*. ^c Crosses of *C. gypsicola*, *C. boissieri*, *C. curdicus* and members of the newly discovered *Femeniasia* to other species of *Carthamus* have not been published to date.

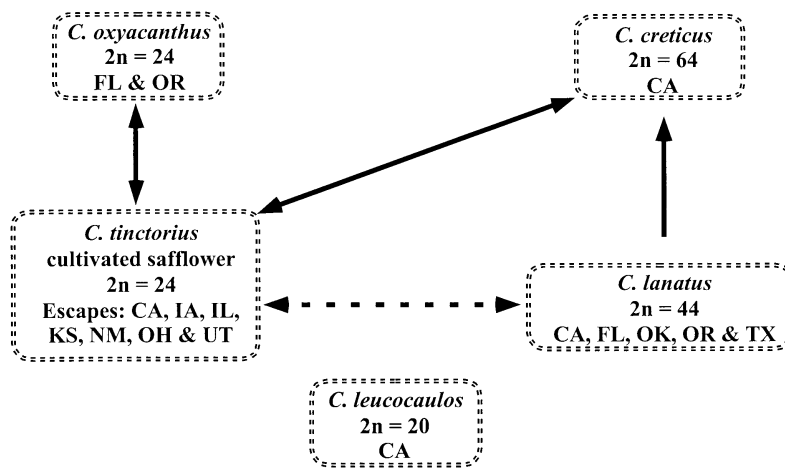


Fig. 2. Summary of interspecific hybridization experiments for taxa found in the New World (see text for references). Solid lines indicate fertile F_1 hybrids with viable seed production. Dotted lines indicate hybridization occurred, but F_1 hybrids could not be obtained without embryo rescue and/or treatments with colchicine. Arrows indicate the direction of the cross (male \rightarrow female). Symbols: CA California, FL Florida, IA Iowa, IL Illinois, KS Kansas, NM New Mexico, OH Ohio, OK Oklahoma, OR Oregon, TX Texas, UT Utah.

Section Atractylis (Polyploid Taxa)

Khidir and Knowles (1970a) suggest that both *C. creticus* ($A_1A_1B_1B_1A_2A_2$) and *C. turkestanicus* ($A_1A_1B_1B_1A_3A_3$) are a product of independent historical hybridization events with an ancestral form of *C. lanatus* ($n = 22$; $A_1A_1B_1B_1$) and

two different species with $n = 10$. Crosses of the hexaploids, *C. turkestanicus* ($n = 32$) and *C. creticus* ($n = 32$) yielded hybrids with several irregularities during meiosis including quadrivalents. The quadrivalent formation of chromosomes during meiosis was used to infer a reciprocal translocation

Table 2. Geographical distributions of *Carthamus sensu* López-González (1989).

Taxon	Known location (endemic or naturalized)
<i>Carthamus</i> L.	
<i>C. curdicus</i> Hanelt	N. Iran only
<i>C. gypsicola</i> Ilj.	N. W. Iran, N. Iraq, S. W. Kazakhstan, Leb., Turkmenistan, Uzbekistan, Syria, and Tur. (Caspian to the Aral Sea)
<i>C. oxyacanthus</i> Bieb.	Aus., Caucasus, S. E. Eur., N. W., India to central Iraq (including Kash.), Iran, Israel, Jordan, S. W. Kazakhstan, Pak., USA (FL & OR), Syria, Turkmenistan, S. Tur., and Uzbekistan
<i>C. palaestinus</i> Eig	S. Israel
<i>C. persicus</i> Willd.	W. Iran, Israel, N. Iraq, Leb., Syria, and Tur.
<i>C. tinctorius</i> L.	Widely cultivated; probable origins in W. Asia ²
<i>Odonthagnathis</i> (DC.) Hanelt	
<i>C. boissieri</i> Halácsy	unknown
<i>C. dentatus</i> (Forssk.) Vahl	Balkans and Aegean region (including Crete, Cyprus and Gr.), Aus., and Tur. to Iran
<i>C. divaricatus</i> Beguinot & Vacc.	Only known from coastal Libya
<i>C. glaucus</i> Bieb.	N. W. Afr. to Egypt along the Mediterranean coast to Israel, Azerbaijan, Tur. to Kash., W. Crete, E. Eur., Iran, Jordan, Leb., Libya, Syria, and S. Ukraine
<i>C. leucocaulos</i> Sm.	Aus., Islands of the Aegean Sea and adjacent Gr. (including Crete), S. E. Eur., S. Afr., Aus., Arg., and USA (CA and OR)
<i>C. tenuis</i> (Boiss. & Bl.) Bornm.	Cyprus, Egypt, Israel, Leb. and S. W. Tur.
Uncertain placement	
<i>C. nitidus</i> Boiss.	Israel, Jordan, Leb., Syria, and Palestine
<i>Atractylis</i> Reichenb.	
<i>C. creticus</i> L.	Aus., Crete, Egypt, Iraq, USA (CA), Rhodes, and Tur.
<i>C. lanatus</i> L.	Tur. to Kash. (Afg.), Canary Islands, S. Eur., N. and S. Afr., Aus., Arg., Chile and USA (CA, FL, OK, OR and TX)
<i>C. turkestanicus</i> Popov	W. Tur. to Kash., Iraq, Pak., Afg., and Eth.

²An extensive evaluation of germplasm from all of the locations of the world that *C. tinctorius* was known to be cultivated was conducted by Ashri et al. (1975). Symbols: Afg. Afganistan, Afr. Africa, Aus. Australia, Arg. Argentina, Eth. Ethiopia, Eur. Europe, Gr. Greece, Kash. Kashmir, Leb. Lebanon, Pak. Pakistan, Tur. Turkey, USA United States of America, CA California, FL Florida, OK Oklahoma, OR Oregon, TX Texas, W. west, E. east, N. north, and S. south.

and thus substantial genetic difference between these species (Khidir and Knowles 1970a). The hybrids of these two hexaploid species produced both pollen and seed with low fertility (Fig. 1; Ashri and Knowles 1960; Khidir and Knowles 1970a). These results substantiate a distant relationship between these two hexaploid species (Table 1), but does not rule out the possibility that an ancestor of *C. lanatus* ($n = 22$) and two different species ($n = 10$) hybridized to produce these distinct polyploid species.

Crosses of *C. lanatus* ($n = 22$) with either *C. creticus* or *C. turkestanicus* ($n = 32$) produced hybrids that formed 22 bivalent and 10 univalent chromosomes during meiosis I (Khidir and Knowles 1970a, b; Estilai and Knowles 1978). The analysis of alcohol dehydrogenase allozymes by Efron et al. (1973) demonstrated that *C. lanatus*, *C. creticus* and *C. turkestanicus* share a unique allele for one subunit of this enzyme not found in other species of *Carthamus*. The results of the hybridization and allozyme studies further substantiate the idea that an ancestor of *C. lanatus* may have been one of the progenitors of the hexaploid ($n = 32$) members of *Carthamus*.

Khidir and Knowles (1970b) hypothesized that *C. creticus* is the product of a hybridization event between taxa similar to the modern *C. leucocaulos* and *C. lanatus*. Crosses with *C. leucocaulos* ($n = 10$) and *C. lanatus* ($n = 22$) produced hybrids ($n = 16$) that were highly similar morphologically to *C. creticus* ($n = 32$), especially when the chromosome number of the resulting hybrids was doubled artificially with colchicine. The

F_1 plants from these crosses had close chromosome homology determined by pairing at meiosis I; however, authors reported that this could be due to autosynditic pairing of chromosomes from the polyploid *C. lanatus* (Ashri and Knowles 1960; Khidir and Knowles 1970a). These hybrids had poor pollen viability and did not produce viable seeds (Ashri and Knowles 1960; Khidir and Knowles 1970a). Geographic ranges of *C. leucocaulos*, *C. lanatus*, and *C. creticus* overlap in Crete, where they are difficult to distinguish morphologically (Table 2; Khidir and Knowles 1970a). Grant noted (1971, pp. 52) that when hybrids and parental species exchange genes frequently, introgressive populations can be created and these populations may lack distinct morphological and ecological characteristics of the original species. The similarities among *C. creticus*, *C. lanatus* and *C. leucocaulos*, where sympatric, are indicative of current or historic gene flow or, alternatively, parallel evolution.

Evidence for the relationship between *C. lanatus* and *C. turkestanicus* comes from the results of hybridization experiments where all of the species with $n = 10$ were crossed with *C. lanatus* (Fig. 1; Khidir and Knowles 1970b). These crosses produced F_1 hybrids with floral heads that resembled those of *C. turkestanicus* ($n = 32$; Khidir and Knowles 1970b). The geographical ranges of *C. turkestanicus* and *C. lanatus* overlap in an area west of Turkey to Kashmir (Table 2). In this area, both species are difficult to distinguish from one another (Khidir and Knowles 1970a), which may be indicative of an introgressive population between two close relatives in an area of sympatry (Grant 1971).

Section Odonthagnathis (Taxa with n = 10 and 11)

A close relationship among the taxa with $n = 10$ was found from crosses between all of these species with one another (Figs. 1 and 2). The close relationship among the taxa with $n = 10$ are reflected in the classification of López-González (1989; Table 1). Hybrids from these crosses were fertile, but crosses of these species with *C. tinctorius* produced sterile hybrids (Fig. 1; Ashri and Knowles 1960; Schank and Knowles 1964). Hybrids from interspecific crosses of *C. divaricatus* ($n = 11$) and species with 20 somatic chromosomes resulted in good chromosome pairing during meiosis I, and partially fertile pollen and seed, which was interpreted as evidence for a close relationship between these taxa (Fig. 1; Estilal and Knowles 1978). Crosses with *C. tinctorius* and *C. divaricatus* produced self-incompatible hybrids that were fertile. The offspring from backcrosses of these hybrids with *C. tinctorius* had low fertility (Fig. 1; Estilal and Knowles 1976).

Estilal and Knowles (1978) placed the species with $n = 10$ into their section II (Table 1) and split this section into two groups based on reciprocal translocations that were observed during meiosis of interspecific crosses among these species. Specific status of several varieties of *C. glaucus* has been proposed (Table 1). The subspecies of *C. glaucus sensu* Hanelt (1963) are all interfertile (Knowles and Schank 1964); however, varieties from Palestine crossed with those from Iran and Syria yield hybrids with one or more translocations during meiosis. These results are indicative of regional variation within *C. glaucus*. The different regional variants of *C. glaucus* do not behave the same when used in interspecific crosses. For example, *C. glaucus* ssp. *glaucus* from Syria and Iran crossed with *C. tenuis* produced fertile and infertile hybrids, respectively (Fig. 1). *Carthamus glaucus* ssp. *alexandrinus* crossed with *C. tinctorius* produced infertile hybrids (Fig. 1; Estilal 1977), but other subspecies or varieties not yet tested may produce viable hybrids with cultivated safflower. *Carthamus glaucus* ssp. *glandulosus* is intermediate to and resembles artificial hybrids of *C. glaucus* ssp. *anatolicus* and *C. tenuis* (Ashri 1973). Where *C. glaucus* ssp. *glandulosus* is sympatric with either *C. glaucus* ssp. *anatolicus* or *C. tenuis* they are difficult to differentiate morphologically (Ashri 1973). These observations and the results of artificial hybridization experiments led Ashri (1973) to conclude that the subspecies of both *C. glaucus* and *C. tenuis* were a single biological species in a hybrid swarm with some divergence over their geographic range. F_1 hybrids were obtained from *Carthamus glaucus* ssp. *alexandrinus* crossed with *C. creticus* (not the reciprocal), and *C. glaucus* ssp. *anatolicus* crossed with *C. lanatus*, but their fertility was not reported (Khidir and Knowles 1970b).

Uncertain Placement (Carthamus nitidus)

Conflicting results have been reported for the relationship of *C. nitidus* with the other species of *Carthamus*. Crosses between *C. tinctorius* ($n = 12$) and *C. nitidus* ($n = 12$) produced sterile F_1 progeny (Fig. 1; Knowles and Schank 1964; Knowles 1989). Crosses of *C. nitidus* with both *C. dentatus* and *C. glaucus* ($n = 10$) failed to produce hybrids (Fig. 1; Knowles and Schank 1964). Based on these hybridization

experiments, Knowles and Schank (1964) suggested that *C. nitidus* was more closely allied to section *Carthamus* ($n = 12$), however, the molecular study of Vilatersana et al. (2000) placed *C. nitidus* as the sister group of section *Odonthagnathis* ($n = 10, 11$; Table 1).

Based on the observed pairing behavior of chromosomes during meiosis of interspecific hybrids, genomic formulas have been assigned to several members of *Carthamus* (Table 3; Khidir and Knowles 1970b; Estilal and Knowles 1978). These genomic formulas provide information about the interspecific compatibility among the genomes of *Carthamus* and may be used to develop hypotheses about the potential for movement of transgenes from interspecific hybrids leading to introgression.

Knowledge of hybridization potential among species of *Carthamus* (Fig. 1) and the species in the New World (Fig. 2) provide information about the biological potential of gene flow from a transgenic safflower variety to wild relatives. The compatibility of cultivated safflower with species from genera closely related to *Carthamus* (*Centaurea*, *Carlina*, *Atractylis*, *Phonus*, and *Carduncellus*) has not been studied extensively and only a few have been mentioned here. The possibility of hybridization with one of these taxa cannot be ruled out without empirical evidence. It should be noted that successful hybridization of two species experimentally (artificially) does not predict success in nature; however, it does establish potential cross-compatibility for hybridization and introgression (Ellstrand et al. 1999). Thus, we can hypothesize that *C. tinctorius* may hybridize with several wild relatives (Figs. 1 and 2) if both species were to flower in synchrony (temporal sympatry) and were growing close enough to one another for wind or insect vectors to transfer pollen between the plants (spatial sympatry).

Geographic Distribution

Members of the genus *Carthamus* (Tour.) L. and *Carduncellus* Adans. are endemic to the eastern and western Mediterranean Basin, respectively (Table 2; Knowles 1969; Vilatersana et al. 2000).

Cultivated varieties of *C. tinctorius* have diverged because of cultivation by man in semi-isolated regions of the Mediterranean. Geographic areas with distinct centers of similarity for cultivated safflower, and the generalized characteristics of these varieties have been described (Knowles 1969; Ashri et al. 1975). Both Knowles (1969) and Ashri et al. (1975) noted that these groupings were based on generalizations and that gene flow by introductions of cultivars from different regions was ongoing and prevents complete divergence. Knowles (1969) and Ashri et al. (1975) both noted that cultivated safflower in India was morphologically homogeneous and suggested that the Indian populations have been isolated from the others for several generations (Knowles and Ashri 1995).

Cultivation of safflower in the New World began in 1899, but it was not commercially grown until the early 1950s (Knowles 1958, 1989). In the United States, *C. tinctorius* has been reported to have escaped cultivation in California (Munz 1968; Hickman 1993), Iowa (Rydberg 1971), Illinois (Henry 1992), Kansas (Gates 1940; Rydberg 1971), New

Table 3. Self-compatibility and genomic formula for the species of *Carthamus sensu* López-González (1989)

Taxon	Self-compatibility ^z	Genomic formula ^y
Section <i>Carthamus</i> (2n = 24)		
<i>C. curdicus</i> Hanelt	Compatible	–
<i>C. gypsicola</i> Ilj.	Compatible	–
<i>C. oxyacanthus</i> Bieb.	Both known	BB
<i>C. palaestinus</i> Eig.	Compatible	B ₁ B ₁
<i>C. persicus</i> Willd. (syn. <i>C. flavescens</i> Spreng.)	Incompatible	B ₁ B ₁
<i>C. tinctorius</i> L.	Compatible	BB
Uncertain placement (2n = 24)		
<i>C. nitidus</i> Boiss.	Compatible	–
Section <i>Odonthognathis</i> (DC.) Hanelt (2n = 20, 22)		
<i>C. boissieri</i> Halácsy	Unknown	–
<i>C. dentatus</i> (Forssk.) Vahl.	Incompatible	A ₁ A ₁
<i>C. divaricatus</i> Begunot & Vacc.	Incompatible	–
<i>C. glaucus</i> Bieb.	Unknown	AAA ₃ A ₃
<i>C. leucocaulos</i> Sm.	Compatible	A ₂ A ₂ ^x
<i>C. tenuis</i> (Boiss. & Bl.) Bornm.	Unknown	–
Section <i>Atractylis</i> Reichenb. (2n = 44, 64)		
<i>C. creticus</i> L.	Compatible	A ₁ A ₁ B ₁ B ₁ A ₂ A ₂
<i>C. lanatus</i> L.	Compatible	A ₁ A ₁ B ₁ B ₁
<i>C. turkestanicus</i> Popov	Compatible	A ₁ A ₁ B ₁ B ₁ A ₃ A ₃
Previously considered members of <i>Carthamus</i> / <i>Carduncellus</i> (2n = 24)		
<i>Lamottea caeruleus</i> (L.) Pomel	Unknown	Unknown
<i>Phonus arborescens</i> (L.) G. López	Unknown	Unknown
<i>Phonus rhiphaeus</i> G. López	Unknown	Unknown

^zWhere tested the incompatibility system has been shown to be of a sporophytic type (Imrie and Knowles 1971; Knowles 1989).

^yGenomic formulas are from Khidir and Knowles 1970b (also see review by Kumar 1991)

^xThe genomic formula for *C. leucocaulos* was changed to AA by Estilai and Knowles (1978).

Mexico (Martin and Hutchins 1981), Ohio (Vincent and Cusick 1998) and Utah (Shaw 1989: Table 2).

Four wild safflower relatives have been introduced in some areas of the New World (Table 2; Hickman 1993). *Carthamus oxyacanthus* ($n = 12$) and *C. lanatus* have been documented as being naturalized in Oregon and Florida (Hickman 1993). *Carthamus lanatus* and *C. leucocaulos* ($n = 20$) have been documented in Texas and have been collected from Argentina and Chile (Correll and Johnston 1970; Marticorena and Quezada 1985; Hickman 1993). *Carthamus creticus* ($n = 32$) and *C. lanatus* ($n = 22$) have been reported in California (Munz 1968; Munz and Keck 1968; Hoover 1970; Hickman 1993). *Carthamus lanatus* has been a weed in California since 1891 (Fuller 1979). Kessler (1987) documented *C. lanatus* as a serious weed growing in an isolated area in Oklahoma.

Biology and Ecology

Information is limited on the biology and ecology of most of the species of *Carthamus*, however, some species have been studied as crops or weeds.

Carthamus tinctorius

Carthamus tinctorius is predominantly a self-compatible weedy annual thistle, with branched upright stems of 30–150 cm in height with terminal flowers, a deep taproot

with laterals, and an inflorescence with a dense capitulum with green ovoid involucral bracts (Smith 1996). Pollination of *C. tinctorius* in Nebraska was facilitated by various insect vectors including *Halictus pictus* (sweat or mining bees), *Agapostemon radiatus* (sweat or mining bees) and *Chauliognathus basalis* (soldier beetles) and in California primarily by *Apis mellifera* (honeybees; Claassen 1950). Smith (1996) stated that *C. tinctorius* does not frequently establish itself outside human cultivated areas; however, feral populations have been documented in several US states (Gates 1940; Rydberg 1971; Martin and Hutchins 1981; Shaw 1989; Henry 1992; Hickman 1993). The longevity of these escapes has not been documented. Knowles (1989) outlined three types of environments suited to growing non-cultivated *C. tinctorius*, all similar to its native Mediterranean region. All three environments were characterized by heavy soils with rains before or just after the seeds are sown followed by a dry period during the later stages of growth.

Carthamus lanatus

Carthamus lanatus ($n = 22$) is a self-compatible, shallow-rooted, annual or facultative biennial (Groves and Kaye 1989), with upright stems from 0.1 m to 2 m in height. It has a rosette of leaves reaching 15 cm in diameter (Kessler

1987; Peirce 1992). Seeds are not developed for wind dispersal; however, the pappus facilitates flotation in water and adherence to animal fur and clothing (Peirce 1992). Pollination of this species is facilitated by honey bees (*Apis mellifera*; Peirce 1992). In Australia, seeds of *C. lanatus* are dispersed by hay, chaff, grain seed contamination and by attachment to sheep wool (Kessler 1987). Australian *C. lanatus* produces 70 to 177 viable seeds per plant (Peirce 1992). Seeds can remain viable for 8 to 10 yr (Quinlivan and Pierce 1969), but most germinate within 3 yr (Peirce 1992). In Oklahoma, *C. lanatus* flowers during the late summer or early fall when *C. tinctorius* is being harvested (Kessler 1987). In California, it flowers between July and August (Kessler 1987). *Carthamus lanatus* establishes in areas where the soil has been disturbed or perennial grasses have been thinned or removed. This species populates areas that have experienced drought or overgrazing (Peirce 1992). It is weedy in pastures and cereal crops in Australia (Peirce 1990).

Carthamus lanatus has been introduced to South Africa, North America and Australia (Peirce 1992). In Australia, it has had control legislation since 1887 (Kessler 1987). In New South Wales, this species has been considered one of the most serious, difficult and costly thistle weeds (Briese 1988), causing yield losses of up to 50 to 70% of cereal crops (Peirce 1992). In Victoria, however, it is not recognized as an aggressive weed (Brooke Thompson, personal communication). The distribution of this species in Australia is predominantly near cereal-growing districts, especially in southeastern regions of the continent (Kessler 1987). In the United States, *C. lanatus* has been identified as a potentially serious weed in Oklahoma, where a single, isolated, population has been difficult to eradicate (Kessler 1987). Cattle and sheep avoid grazing on *C. lanatus* (Kessler 1987; Peirce 1992).

Seed Biology of Some *Carthamus* Species

Successful introduction and naturalization of a *Carthamus* species in the New World depends on their ability to reach maturity, reproduce within the growing season and whether the seeds can survive adverse environmental conditions.

Some research has been published on the seed biology of some species of *Carthamus*, which may provide insights into the longevity and survivorship in the environment. Kessler (1987) determined that germination of *C. lanatus* seeds were reduced or prevented by moisture stress (limited at -0.25 MPa and no germination at -0.5 MPa) and were long-lived in the soil. The optimal germination conditions for seeds of *C. lanatus* were found to be 8 h at 20°C and 16 h at 10°C (Groves and Kaye 1989). Seeds of *C. lanatus* were unaffected by freezing at -16°C while other thistle species (not members of *Carthamus*) were injured. Experimental varieties of *C. tinctorius* have been able to withstand temperatures as low as -15°C following a hardening period (Zimmerman and Buck 1977; Knowles and Ashri 1995). Seeds of *C. persicus* were able to withstand temperatures of -16°C for 4 h with about 50% of the seeds remaining viable. Crosses with *C. tinctorius* and *C. persicus* produced hybrid plants with cold tolerance similar to the latter species

(Zimmerman and Buck 1977). Gupta and Murty (1986) reported seeds of *C. oxyacanthus* to be resistant to low and high humidity and both high and fluctuating temperatures. The ability of seeds from *C. tinctorius* and its wild relatives to withstand extreme environmental conditions is not well understood and requires further research.

Outcrossing Rates

Cross pollination of safflower plants is facilitated predominantly by insects, and wind can move pollen short distances (up to ca. 122 cm) between plants grown close together (Claassen 1950; Ashri and Rudich 1965). Bees and other flying insects may contribute to gene flow among *C. tinctorius* and its wild relatives over relatively large distances (Kadam and Patankar 1942; Claassen 1950). Claassen (1950) grew (safflower) plants in close proximity (less than 122 cm) and found levels of outcrossing that varied from 5 to 45% in some cultivars, but most cultivars were found to have less than 10% outcrossing (Kadam and Patankar 1942; Claassen 1950; Knowles 1969). The amount of outcrossing was found to be highly variable among safflower cultivars and was partially controlled by heredity (Claassen 1950). Among individual plants from various safflower lines the range in cross pollination was 0 to 100% (Claassen 1950). The variation in outcrossing rates among different cultivars of *C. tinctorius* (Claassen 1950) means that empirical measurements of outcrossing frequency should be conducted under different environmental conditions for cultivars of interest. The variation in outcrossing rate between cultivars means that this value should be experimentally determined for cultivars being considered for the production of plant-made pharmaceuticals.

Ecological Niche

The niche occupied by a wild crop relative is often indicative of the spatial relationship between a wild crop relative and crop and, thus, the potential for introgression. *Carthamus tinctorius* is the product of selection by humans in an agricultural environment, whereas *C. palaestinus*, *C. persicus* (syn. *C. flavescens*) and *C. oxyacanthus* are considered weeds in areas disrupted by human and agricultural activities (Baker 1965; Imrie and Knowles 1970). *Carthamus tinctorius* has a shorter duration of the rosette stage selected to enhance earlier maturity of the crop. In addition, the seeds of *C. tinctorius* are shatter resistant and generally lack a pappus, ensuring that seeds are easily harvested (Ashri and Efron 1964). *Carthamus persicus* is a weed of wheat fields, pastures and roadsides of cooler climates in eastern Iraq, Lebanon, Syria and Turkey (Imrie and Knowles 1970; Knowles and Ashri 1995; Smith 1996). Its weediness is attributed to a delayed rosette stage with aerial stem and flower development after the harvest of cereal crops, thus ensuring its seeds are distributed back to the field. The seeds of *C. persicus* are easily released by shattering and have a pappus, both traits advantageous for wind dispersal. The most broadly distributed weedy species of *Carthamus* in the Mediterranean is *Carthamus oxyacanthus*. It has seeds that are released by shattering, but the seeds have a reduced pappus (Ashri and Efron 1964). This species is found in subtropical regions of western Iraq, Iran,

northwest India, throughout Kazakhstan, Turkmenistan, and Uzbekistan (Table 2; Smith 1996; Knowles and Ashri 1995). *Carthamus palaestinus* has seeds that are released by shattering (Ashri and Efron 1964) and is known only from desert regions of western Iraq, Israel, and Jordan (Knowles and Ashri 1995; Smith 1996).

CONCLUSION

A transgenic safflower variety (*Carthamus tinctorius* L.) has been derived by the fusion of a gene encoding a protein based pharmaceutical with the protein oleosin. The purification of a protein from the mature oil rich seeds (ca. 30–40% of the seed dry weight) of safflower has the potential for economic success (Smith 1996) and the isolation of oleosin from safflower seed has been documented (Lacey et al. 1998). A concern with this new technology is the escape of transgenes into the environment. Gene flow from the crop to one of its wild relatives could lead to introgression, the stable incorporation of a transgene. Direct environmental consequences include the loss of herbicidal control of weed relatives and therefore their potential increase. More importantly, there is a public perception that transgenes pose unknown risks and therefore must be contained.

Most domesticated plants have been reproductively isolated from their natural populations for less than ca. 1000 generations (Ellstrand et al. 1999). In this short time, it is unlikely that complete reproductive isolation has occurred. Gene flow at low rates among populations can reduce differentiation generated by genetic drift, mutations, and natural selection, which in turn prevents divergence of populations from one another (Arnold 1997; Ellstrand et al. 1999). Although *Carthamus tinctorius* has been cultivated for more than 1000 yr (Knowles and Ashri 1995; Smith 1996), interspecific hybridization experiments have shown that *C. tinctorius* can be crossed with several wild relatives to produce fertile progeny (Figs. 1 and 2; Ashri and Knowles 1960; Schank and Knowles 1964; Khidir and Knowles 1970a, b; Imrie and Knowles 1970, 1971; Estilai and Knowles 1978; Heaton and Klisiewicz 1981; reviewed by Kumar 1991).

Potential recipients of nuclear encoded transgenes from a cultivated plant-made pharmaceutical with safflower in the New World include *C. tinctorius* escapes from cultivation and four naturalized wild relatives (*C. creticus*, *C. lanatus*, *C. leucocaulos* and *C. oxyacanthus*). Of these wild species, only *C. oxyacanthus* and *C. creticus* have been shown to produce viable hybrid offspring when crossed with cultivated *C. tinctorius* and then only under some conditions (Figs. 1 and 2). Hybrids of *C. tinctorius* and *C. lanatus* were not viable without the use of embryo rescue and chromosome doubling techniques (Fig. 1; Ashri and Knowles 1960; Heaton and Klisiewicz 1981). Thus, this hybrid is not likely to survive outside a laboratory setting. *Carthamus tinctorius* has been naturalized in parts of the United States, thus making it another potential transgene recipient and a possible intermediary for transgene movement to wild species. However, the frequency of gene exchange would depend on the outcrossing frequency of both naturalized safflower cultivars and the transgenic crop. Gene

flow has the potential to occur between a transgenic crop of safflower (*C. tinctorius*) and either *C. oxyacanthus* or *C. creticus* in several regions of the New World where these species are sympatric, including most regions of the New World currently utilized to cultivate safflower except in the prairie provinces of southern Canada (Fig. 2; Table 2).

Mitigation of the potential for transgene escape can best be achieved by avoiding the cultivation of a transgenic *C. tinctorius* in areas where feral safflowers occur and when cross compatible weedy relatives are suspected. The lack of wild and weedy relatives of cultivated *C. tinctorius* in the western prairie regions of Canada may provide a safe region to grow a plant-made pharmaceutical safflower compared with more southern locations in the New World. Some regions in the southern US could also provide isolated locations for the cultivation of transgenic safflower. Cultivation of this new crop is not suitable in Asia and the Middle East where several wild and weedy relatives are thought to currently hybridize frequently with cultivated safflower (Deshpande 1952; Ashri and Rudich 1965; Knowles and Ashri 1995).

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